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A Novel 105 Basepair Deletion Causing β^0-Thalassemia in Members of a Thai Family

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We identified and characterized a novel β^0-thalassemia mutation due to partial deletion of the 5′ end β-globin gene including the mRNA cap site and a part of exon 1. The deletion was precisely 105 basepair (bp) in length extending from position –24 or –25 to +80 or +81 relative to the β-globin gene mRNA cap site. This mutation was detected in three individuals from a family originating in the area of southern Thailand. The propositus was a 39-year-old female and noted to be heterozygous for β-thalassemia with hemoglobin (Hb) level of 10.1 g/dl, MCV 70 fl, MCH 23.1 pg, HbA_2 6.3%, and HbF 2.4%. Her son was 9 years of age and was also heterozygous for the mutation, having Hb level of 10.8 g/dl, MCV 58 fl, MCH 19.0 pg, HbA_2 5.6%, and HbF 4.3%. Her 6-year-old daughter was affected, having a genotype of this mutation and a G-C transition at IVS 1 nt 5. Although the deletion does not include the β-globin gene promoter sequences, the individuals heterozygous for this mutation have an elevated HbA_2 level slightly higher than observed in most carriers of β-thalassemia caused by point mutations. Am. J. Hematol. 61:1–4, 1999.

Key words: beta-thalassemia; deletional mutation; Thai family

INTRODUCTION

The prevalence of β-thalassemia in Thailand varies from 3 to 9% with more than 20 known mutations [1–5]. The mutations are heterogenous and mainly caused by single base substitutions and insertions or deletions of a few nucleotides. Only two mutations caused by large deletions of the β-globin gene have been detected. They are due to 619 basepair (bp) deletion of the 3′ end that is the Asian Indian type [2,6] and 3485 bp deletion of the entire β-globin gene including the promoter region, which has originally been described in Thai [7,8].

In this study, we report a new deletion in the 5′ β-globin gene region detected in β-thalassemia subjects from a Thai family. The deletion is 105 bp in length with the 5′ end at position –24 or –25 and the 3′ end at position +80 to +81 relative to the β-globin gene mRNA cap site. Although the deletion does not remove the β-globin gene promoter TATA, CCAAT and CACCC boxes that are involved in regulation of transcription [9–11], subjects heterozygous for this mutation have an HbA_2 level slightly higher than observed in other subjects heterozygous for point mutations.

MATERIALS AND METHODS

Peripheral blood samples were collected from the subjects using EDTA as anticoagulant. Blood count and erythrocyte indices were determined using an automated blood cell counter (Technicon H*1E™ system, Miles Inc., Tarrytown, NY). Hb electrophoresis was performed on cellulose acetate membrane pH 8.6. HbA_2 and HbF levels were quantified by DEAE Sephadex A-50 column chromatography [12].

Genomic DNA was prepared from the buffy coat layer of EDTA peripheral blood samples by phenol/chloroform extraction [13]. The β-globin gene was amplified by the polymerase chain reaction (PCR) procedure [14] using primers S1: 5′ TGT CAT CAC TTA GAC CTC

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TABLE I. Hematological Study and DNA Analysis of Members of 105-bp Mutation Family*

Subject	Age/sex	Relation	Hb (g/dL)	MCV (fl)	MCH (pg)	Hb type	HbA_2[a] (%)	HbF[a] (%)	Genotype
1	39/F	Propositus	10.1	70	23.1	AA2	6.3	2.4	105-bp/N
2	34/M	Husband	13.8	67	21.2	AA2	4.5	1.7	IVS1#5/N
3	9/M	Son	10.8	58	19.0	AA2	5.6	4.3	105-bp/N
4	6/F	Daughter	2.5	59	21.9	FA2	4.8	95.2	105-bp/IVS1#5
5	4/M	Son	9.1	58	19.0	AA2	4.7	2.1	IVS1#5/N

*Hb, hemoglobin; MCV, mean corpuscular volume; N, Normal.
[a]By DEAE Sephadex A-50 column chromatography.

AC 3′ located at positions –122 to –103 relative to the β-globin gene mRNA cap site and S3: 5′ TCC CAT AGA CTC ACC CTG AA 3″ was complementary to the sense strand and located 631 bp downstream from primer S1 [1]. The deletion and normal fragments were isolated from an agarose gel and sequenced directly using primer G3: 5′ GCC CAG TTT CTA TTG GTC TC 3′ at positions +174 to +193 relative to the cap site and the dideoxy chain termination method [15] using a T7 sequencing kit (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). The breakpoints of the deletion were determined by comparing the nucleotide sequence of the mutant allele with the normal one in the region of deletion.

The presence of deletion was confirmed by dot blot hybridization of S1S3-PCR fragments to the digoxigenin-labeled oligonucleotide probes CN1: 5′ GCA TAA AAG CCG TTA CTG 3′ for the deletion fragment and CN2: 5′ GCA TAA AAG TCA GGG CAG 3′ for the normal fragment. The hybridized DNA probes were detected with antidigoxigenin-alkaline phosphatase system (DIG oligonucleotide 3′-end labeling kit and Digoxigenin Detection kit, Boehringer Mannheim, Germany).

RESULTS

The propositus was a healthy 39-year-old Thai female who had mild anemia with hypochromic and microcytic red cells (Hb 10.1 g/dl, MCV 70 fl, and MCH 23.1 pg). The levels of HbA_2 and HbF were 6.3% and 2.4%, respectively. The same condition was found in her 9-year-old son with Hb 10.8 g/dl, MCV 58 fl, MCH 19.0 pg, HbA_2 5.6%, and HbF 4.3%. Hematological data of members of this family are shown in Table I.

The β-globin gene was amplified by PCR using the primers that lie 631 bp covering the deletion region. The deleted and normal β-globin gene alleles yielded amplified products of ~500 and 631 bp, respectively (Fig. 1). The small fragment was isolated and subsequently analyzed by DNA sequencing. The results showed that the deletion was precisely 105 bp in length, extending from position –25 or –24 to +80 or +81 relative to the β-globin gene mRNA cap site (Fig. 2). Two breakpoints are possible because of the presence of one guanine nucleotide at both deletion ends. These findings were confirmed by dot blot hybridization of the amplified DNA samples with allele-specific oligonucleotide probes designed to detect the mutation. The hybridization result is shown in Fig. 3. PCR fragments from the propositus, her daughter, and her 6-year-old son were hybridized to probes CN1 which was specific for the deleted fragment and CN2 specific for normal fragment. They are all heterozygous for this new mutation, whereas their father and 4-year-old son are normal.

Blood samples taken from the members of this family—father, mother, one daughter, and two sons—were analyzed by the above protocols. The results are shown in Table I.

DISCUSSION

Recently, nearly 200 mutations have been characterized to produce β-thalassemia, which are mostly caused by point mutations [16]. There are 10 deletional forms of β-thalassemia that have been reported, ranging from 44–12622 bp [17–19]. In nine of them, the deletion regions have in common the 5′ β-gene promoters, which exhibited an unusually high level of HbA_2 in the heterozygous state. Several hypotheses have been proposed to explain the increased expression of the δ-globin gene observed in these deletions, such as altered chromatin structure, 3′ β-globin gene enhancer juxtaposition, altered competition of the remaining γ- and δ-globin gene promoters for limited transcription factors or altered competition of the γ-, δ-, and β-globin gene promoters for the LCR sequences [17].

In this study, we report the characterization of a β^0-thalassemia mutation resulting from deletion of 105 bp at the 5′ end β-globin gene in three members of one Thai family. This new deletion removes 25 nucleotides upstream and 80 nucleotides downstream from the mRNA cap site. Although this region does not include the β-globin gene promoters (CACCC, CCAAT, and TATA boxes), the HbA_2 value (5.6%, 6.3%) of individuals heterozygous for this mutation is considerably higher than that observed in most carriers of β-thalassemia mutation

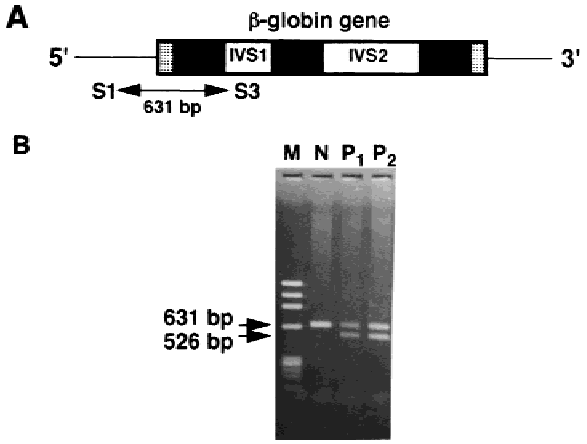

Fig. 1. PCR amplification of the 5′ end of the β-globin gene. (A) A schematic diagram representing the β-globin gene and locations of amplification primers S1 and S3. The 631-bp fragment was obtained from the normal allele. (B) Ethidium bromide stained agarose gel: M, ϕX174 RF DNA/ Hae III marker; N, normal individual; P1, propositus; and P2, her son. The normal and deleted fragments are indicated by arrows.

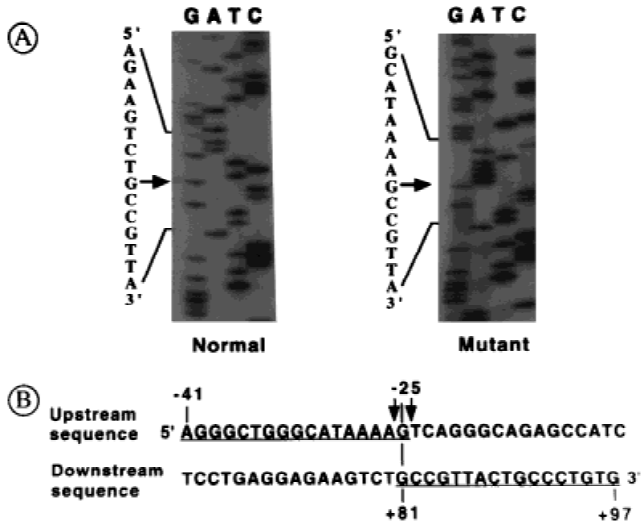

Fig. 2. DNA analysis of the β-thalassemia deletion. (A) DNA sequence autoradiograph for the sense strands across the breakpoint of the 105-bp deletion indicated by arrows. (B) Nucleotide sequences of the junction fragment. Sequences of the deletion allele are underlined. The positions of the 5′ and 3′ sequences are given relative to the cap site of the β-globin gene. Sequence homology is indicated by the vertical lines. The two possible sites for the crossover are indicated by arrows.

caused by base substitutions or small insertions/deletions. From our studies, the HbA_2 level in normal individuals varies from 2.0% to 3.3% with an average of 2.6%, whereas levels for β-thalassemia heterozygotes for various point mutations varies from 3.9 to 6.2% with an average of 5.1%. In comparison with a Thai 3.5 kb deletion, which removes the 5′ β-globin gene including the promoter sequences, the average HbA_2 level for 10 persons is 6.4% with a range of 5.5 to 7.8%. However, the HbA_2 level of the subject heterozygous for this 105 bp deletion is not unusually high as found in other relatively large deletions of the 5′ β-globin gene region [16,17]. These findings suggest that the loss of not only the promoter sequences but also the region 3′ to the TATA box of the β-globin gene affects the transcription of the δ-globin gene with variable efficiency probably because of impaired interaction of LCR with the β-globin locus. There is no obvious sequence homology between 5′ and 3′ regions around the breakpoint, indicating that the deletion may be due to a nonhomologous recombinant event.

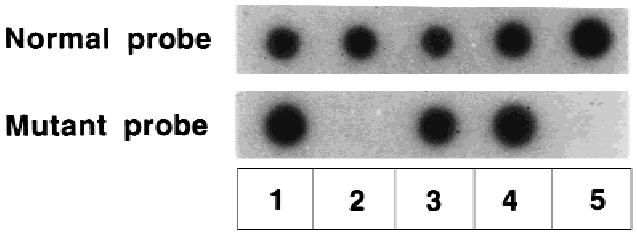

Fig. 3. Dot blot analysis of the amplified DNA samples using the allele-specific oligonucleotide probe for the 105 bp deletion. 1, Propositus; 2, her husband; 3, her 9-year-old son; 4, her 6-year-old daughter; and 5, her 4-year-old son.

Because the deletion is located within the 631-bp region of the PCR fragment obtained from primers S1 (5′ TGT CAT CAC TTA GAC CTC AC 3′) and S3 (5′ TCC CAT AGA CTC ACC CTG AA 3′), it can be detected directly by gel electrophoresis and ethidium bromide staining after the PCR amplification. This new mutation and a simple method for detection will be helpful in planning a prenatal diagnosis program and genetic counseling for the disease.

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